

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
28 April 2005 (28.04.2005)

PCT

(10) International Publication Number  
WO 2005/038039 A3

(51) International Patent Classification:  
C12Q 1/68 (2006.01)

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(21) International Application Number:  
PCT/US2004/033818

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(22) International Filing Date: 13 October 2004 (13.10.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/510,762 13 October 2003 (13.10.2003) US

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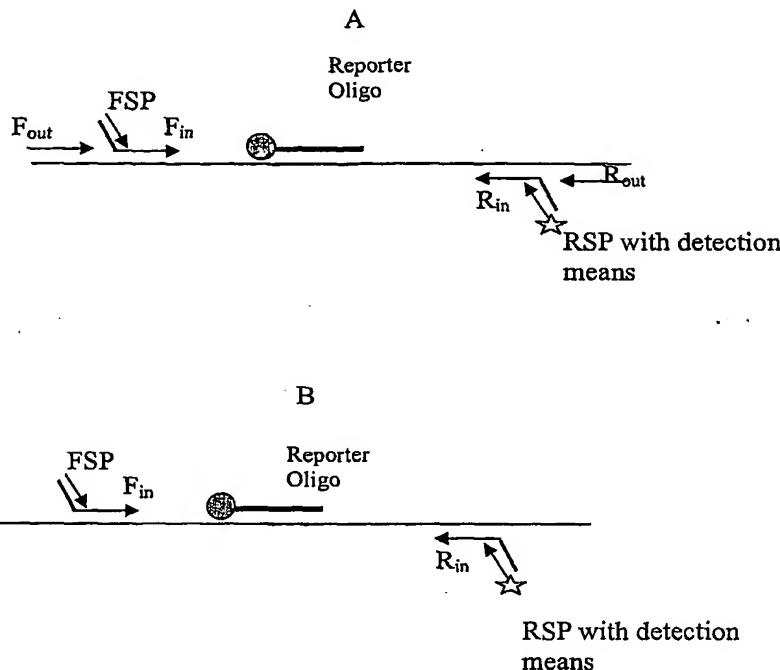
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Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

[Continued on next page]

(54) Title: METHOD FOR MULTIPLEX PRIMER BASED AMPLIFICATION OF NUCLEIC ACIDS



(57) Abstract: Disclosed is a novel method for diagnosis or differential diagnosis of disease agents and secondary disease agents. The method disclosed uses a novel amplification strategy termed TemPCR to allow sensitive and specific amplification of target sequences from any disease agents and/or secondary disease agent whose nucleic acid sequence is known. The TemPCR method utilizes at least one set of target enrichment primers specific for the disease agent or secondary disease agent to be detected (present at a low concentration) and at least one pair of shared target amplification primers (present at high concentrations). At least one pair of said target enrichment primers comprises a binding sequence for the target amplification primers. Therefore, the use of the TemPCR method allows multiplex amplification reactions to be carried out without the need for empirical optimization of the multiplex amplification parameters. Methods

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for nucleic acid isolation and the detection of the target sequences are also disclosed for use with the TemPCR method.



**Published:**

- *with international search report*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

**(88) Date of publication of the international search report:**

28 September 2006

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US04/33818

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC: C12Q 1/68( 2006.01)

USPC: 435/6

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Continuation Sheet

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2003/0096277 A1 (CHEN) 22 May 2003 (22.05.2003), especially claims 10 & 11, Figure 1, p. 8 paragraph 0062, and p. 7 paragraph 0072.	1-3, 8-11, 13-16, 22, 23, 28-34, and 36 -----
X, E --- Y,E	US 2005/0175996 A1 (CHEN) 11 August 2005 (11.08.2005), especially claims 13 and 17, and Figures 2A and 2B.	4-7, 12, 17, and 35 1-3, 8-11, 13-16, 22, 23, 28-34, and 36 -----
Y	QIAGEN. Taq PCR Handbook, March 2002 (03.2002), especially p. 30.	4-7, 12, 17, and 35 4-7, 12, and 17
Y	US 5,194,300 (CHEUNG) 16 March 1993 (16.03.1993), Abstract.	35

Further documents are listed in the continuation of Box C.

See patent family annex.

Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

12 July 2006 (12.07.2006)

Date of mailing of the international search report

11 AUG 2006

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**INTERNATIONAL SEARCH REPORT**

International application No.

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**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.: 18-21  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
Claim to a "a selective amplification process" is unclear and no meaningful search can be performed.
  
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of any additional fees.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-17 and 22-36

**Remark on Protest**

The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**

International application No.  
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**BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING**

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-23 and 28-36, drawn to methods for multiplex primer-based amplification in a reaction mixture comprising at least a first and second pair of target enrichment primers and at least a first pair of target amplification primers.

Group II, claims 37-68, drawn to methods for multiplex primer-based amplification of at least one target sequence carried out in a reaction mixture comprising at least a first pair of target enrichment primers and at least a first pair of target amplification primers.

Group III, claims 69-78, drawn to methods of diagnosing the presence of a disease in a subject comprising providing a sample and isolating a nucleic acid containing a target sequence which is subject to multiplex primer-based amplification.

Group IV, claims 79-88, drawn to methods for differentially diagnosing the presence of a disease agent and a secondary disease agent in a subject comprising providing a sample and isolating a nucleic acid containing a target sequence which is subject to multiplex primer-based amplification.

It is noted that claims 24 through 27 are not included in any group for the following reasons. Claim 24 improperly depends from itself and thus the dependency is unclear. Claims 25 through 27 depend from claim 24 and it is unclear what these claims are depending from.

1. This International Searching Authority considers that the international application does not comply with the requirements of unity of invention (Rules 13.1, 13.2, 13.3 ) for the reasons indicated below:

The inventions listed as Groups I-IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature linking Groups I-IV appears to be that they all relate to multiplexing amplification reactions. However, WO 01/94634 A2 (01-Jun-2001) teaches multiplexing amplification reactions using primers.

Therefore, the technical feature linking the inventions of Groups I-IV does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

Accordingly, Groups I-IV are not so linked by the same or a corresponding special technical feature as to form a single general inventive concept. Please See Continuation Sheet.

**INTERNATIONAL SEARCH REPORT**

International application No. PCT/US04/33818
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Continuation of B. FIELDS SEARCHED Item 3:  
US-PUB, USPAT, EPO, DERWENT, terms searched: primer?, nest?, PCR, and multiplex; inventors searched: Xianging Chen